

AMENDMENTS TO THE CLAIMS

The listing of claims will replace all prior versions, and listings, of claims in the application.

Marked-up Listing of Claims:

1. (Currently Amended) A method for ~~the preparation~~ producing an ~~of~~ evolved microorganism[[s]], comprising ~~the following steps~~:
 - a) ~~preparing~~ generating a ~~modified microorganism by~~ directed genetic modification in a gene of interest in ~~of cells of~~ an initial microorganism to yield a modified microorganism ~~so as to inhibit~~ wherein the production or consumption of a ~~metabolite substrate is inhibited~~ when ~~that~~ the modified microorganism is grown on a defined medium, ~~thereby~~ impairing the ability of ~~that~~ the modified microorganism to grow;
 - b) culturing the modified microorganism ~~thereby~~ obtained in step (a) on said defined medium, allowing the modified microorganism to evolve a compensatory metabolic pathway to compensate for the impaired growth, wherein the defined medium can contain a co-substrate promoting the evolution; and
 - c) selecting a ~~modified~~ an evolved microorganism from step (b) able to grow on said defined medium, ~~if necessary with a co-substrate, and thereby preparing an evolved microorganism;~~
wherein a compensatory metabolic pathway is evolved allowing the evolved microorganism to proliferate on the defined medium.
2. (Currently Amended) The method as claimed in claim 1, wherein the modified microorganism has a modified metabolic pathway comprising ~~selected from the group consisting of:~~ biosynthesis pathways of amino acids, synthesis pathways of nucleic acids, synthesis pathways of lipids, ~~and~~ or metabolism pathways of sugars.
3. (Original) The method as claimed in claim 2, wherein the modified metabolic pathway is a biosynthesis pathway of amino acids.

4. (Previously Presented) The method as claimed in claim 3, wherein the modified metabolic pathway is a biosynthesis pathway of an amino acid chosen from among: methionine, cysteine, threonine, lysine, and isoleucine.
5. (Original) The method as claimed in claim 2, wherein the modified metabolic pathway consumes NADPH.
6. (Original) The method as claimed in claim 1, wherein the modification made in step a) favors the reduction of NADP to NADPH.
7. (Original) The method as claimed in claim 2, wherein the modification made in step a) favors the reduction of NADP to NADPH.
8. (Currently Amended) The method as claimed in claim 1, wherein the evolved microorganism possesses at least one evolved gene coding for an evolved protein, the evolution of which ~~replaces~~ allows the inhibited ~~metabolic pathway~~ growth to be compensated by a new the evolved metabolic pathway.
9. (Original) The method as claimed in claim 8, wherein it includes a step d) consisting of the isolation of the evolved gene coding for the evolved protein.
10. (Cancelled)
11. (Currently Amended) The method as claimed in claim 9, wherein the evolved gene is introduced, ~~in an appropriate form,~~ into a production microorganism intended for the production of the evolved protein.
12. (Cancelled)
13. (Currently Amended) The method as claimed in claim 22 [[12]], further comprising a step d) consisting of the isolation of the evolved gene coding for the evolved protein.
14. (Original) The method as claimed in claim 13, wherein the evolved gene is introduced, in an appropriate form, into a production microorganism intended for the production of the evolved protein.
- Claims 15-21 (Cancelled).
22. (New) The method of claim 1, wherein the gene of interest is a homologous gene or a heterologous gene.

23. (New) The method of claim 1, wherein the defined medium is substantially free of the substrate the production or consumption of which is inhibited in the modified microorganism.
24. (New) The method of claim 1, wherein, in step (c) a co-substrate is added to the defined medium.
25. (New) The method of claim 1, wherein the evolved compensatory pathway includes at least one evolved protein encoded by a homologous gene or a heterologous gene.
26. (New) The method of claim 1, wherein the genetic modification comprises the directed mutation or deletion of a gene of interest or the directed modification of a promoter in said gene of interest.
27. (New) The method of claim 1, wherein the genetic modification consists in the removal of most of the gene of interest.
28. (New) The method of claim 1, wherein the gene of interest is replaced with a selection marker gene.
29. (New) The method of claim 1, wherein the microorganism is selected among bacteria, yeasts and fungi.
30. (New) The method of claim 29, wherein the microorganism is *Aspergillus sp.*, *Bacillus sp.*, *Brevibacterium sp.*, *Clostridium sp.*, *Corynebacterium sp.*, *Escherichia sp.*, *Gluconobacter sp.*, *Pseudomonas sp.*, *Rhodococcus sp.*, *Saccharomyces sp.*, *Streptomyces sp.*, *Xanthomonas sp.*, or *Candida sp.*
31. (New) The method of claim 30, wherein the microorganism is *E. coli* and *C. glutamicum*.
32. (New) A modified microorganism made by the method of claim 1.

Clean Listing of Claims:

1. A method for producing an evolved microorganism, comprising:
 - a) generating a directed genetic modification in a gene of interest in an initial microorganism to yield a modified microorganism wherein the production or consumption of a substrate is inhibited when the modified microorganism is grown on a defined medium, impairing the ability of the modified microorganism to grow;
 - b) culturing the modified microorganism obtained in step (a) on said defined medium, allowing the modified microorganism to evolve a compensatory metabolic pathway to compensate for the impaired growth, wherein the defined medium can contain a co-substrate promoting the evolution; and
 - c) selecting an evolved microorganism from step (b) able to grow on said defined medium; wherein a compensatory metabolic pathway is evolved allowing the evolved microorganism to proliferate on the defined medium.
2. The method as claimed in claim 1, wherein the modified microorganism has a modified metabolic pathway comprising: biosynthesis pathways of amino acids, synthesis pathways of nucleic acids, synthesis pathways of lipids, or metabolism pathways of sugars.
3. The method as claimed in claim 2, wherein the modified metabolic pathway is a biosynthesis pathway of amino acids.
4. The method as claimed in claim 3, wherein the modified metabolic pathway is a biosynthesis pathway of an amino acid chosen from among: methionine, cysteine, threonine, lysine, and isoleucine.
5. The method as claimed in claim 2, wherein the modified metabolic pathway consumes NADPH.
6. The method as claimed in claim 1, wherein the modification made in step a) favors the reduction of NADP to NADPH.
7. The method as claimed in claim 2, wherein the modification made in step a) favors the reduction of NADP to NADPH.

8. The method as claimed in claim 1, wherein the evolved microorganism possesses at least one evolved gene coding for an evolved protein, the evolution of which allows the inhibited growth to be compensated by the evolved metabolic pathway.
9. The method as claimed in claim 8, wherein it includes a step d) consisting of the isolation of the evolved gene coding for the evolved protein.
11. The method as claimed in claim 9, wherein the evolved gene is introduced, into a production microorganism intended for the production of the evolved protein.
13. The method as claimed in claim 22, further comprising a step d) consisting of the isolation of the evolved gene coding for the evolved protein.
14. The method as claimed in claim 13, wherein the evolved gene is introduced, in an appropriate form, into a production microorganism intended for the production of the evolved protein.
22. The method of claim 1, wherein the gene of interest is a homologous gene or a heterologous gene.
23. The method of claim 1, wherein the defined medium is substantially free of the substrate the production or consumption of which is inhibited in the modified microorganism.
24. The method of claim 1, wherein, in step (c) a co-substrate is added to the defined medium.
25. The method of claim 1, wherein the evolved compensatory pathway includes at least one evolved protein encoded by a homologous gene or a heterologous gene.
26. The method of claim 1, wherein the genetic modification comprises the directed mutation or deletion of a gene of interest or the directed modification of a promoter in said gene of interest.
27. The method of claim 1, wherein the genetic modification consists in the removal of most of the gene of interest.
28. The method of claim 1, wherein the gene of interest is replaced with a selection marker gene.

29. The method of claim 1, wherein the microorganism is selected among bacteria, yeasts and fungi.

30. The method of claim 29, wherein the microorganism is *Aspergillus sp.*, *Bacillus sp.*, *Brevibacterium sp.*, *Clostridium sp.*, *Corynebacterium sp.*, *Escherichia sp.*, *Gluconobacter sp.*, *Pseudomonas sp.*, *Rhodococcus sp.*, *Saccharomyces sp.*, *Streptomyces sp.*, *Xanthomonas sp.*, or *Candida sp.*

31. The method of claim 30, wherein the microorganism is *E. coli* and *C. glutamicum*.

32. A modified microorganism made by the method of claim 1.